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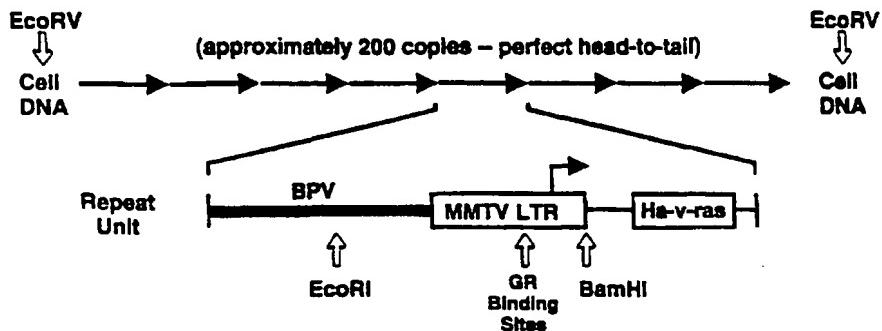
**WORLD INTELLECTUAL PROPERTY ORGANIZATION**  
**International Bureau**



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

**(54) Title: METHOD AND COMPOSITIONS FOR MONITORING DNA BINDING MOLECULES IN LIVING CELLS**

## Structure of the MMTV tandem array in Cell Line 3134



**(57) Abstract**

The present invention provides a method of screening for a compound that binds to a selected nucleic acid comprising contacting compound fluorescently labeled by a fluorescent protein with a cell having a plurality of copies of the nucleic acid in an array such that the nucleic acid can be directly detected when bound by fluorescently labeled compound; and directly detecting the location of fluorescence within the cell, fluorescence aggregated at the site of the nucleic acid array indicating a compound that binds to the selected nucleic acid. In particular compounds such a transcription factor can be screened. Reagents for such method are provided including a mammalian cell having a plurality of steroid receptor response elements in an array such that the response element can be directly detected when bound by fluorescently labeled steroid receptor and a chimeric protein comprising a fluorescent protein fused to a steroid receptor.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/19516

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 6 C12N15/12 C12N15/62 C12N5/10 C07K14/435 C07K14/72  
 C12Q1/02 G01N33/50 G01N33/52

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 6 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROC. NATL.ACAD SCI., vol. 92, no. 25, 5 December 1995, NATL. ACAD SCI., WASHINGTON, DC, US; pages 11899-11903, XP002029556 H. OGAWA ET AL.: "Localization, trafficking, and temperature-dependence of the Aequorea green fluorescent protein in cultured vertebrate cells" cited in the application see the whole document ---	29, 30, 39, 41-44, 46, 48, 51-54, 57
Y	-/-	45, 49

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*'A' document defining the general state of the art which is not considered to be of particular relevance
- \*'E' earlier document but published on or after the international filing date
- \*'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*'O' document referring to an oral disclosure, use, exhibition or other means
- \*'P' document published prior to the international filing date but later than the priority date claimed

- \*'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*'&' document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1997	25.04.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax (+31-70) 340-3016	Authorized officer  Hornig, H

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## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 96/19516

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	THIRTY-FIFTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, WASHINGTON, D.C., USA, DECEMBER 9-13, 1995. MOLECULAR BIOLOGY OF THE CELL 6 (SUPPL.). 1995. 313A. ISSN: 1059-1524, November 1995, XP000670313 MACARA I G ET AL: "Real-time detection of ligand-induced nuclear transport using a glucocorticoid receptor - green fluorescent protein fusion construct." abstract no. 1818 see abstract	29,30, 37,38, 41-46, 48, 51-54,57
Y		49
X	THIRTY-FIFTH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR CELL BIOLOGY, WASHINGTON, D.C., USA, DECEMBER 9-13, 1995. MOLECULAR BIOLOGY OF THE CELL 6 (SUPPL.). 1995. 232A. ISSN: 1059-1524, November 1995, XP002029557 HTUN H ET AL: "GFP - GR: A model system for studying cytoplasm-to-nuclear translocation and nuclear architecture in cultured living cells." abstract no. 1345 see abstract	29,30, 39, 41-46, 48, 51-54,57
Y		49
Y	NATURE, vol. 373, 23 February 1995, MACMILLAN JOURNALS LTD., LONDON,UK, pages 663-664, XP002029558 R. HELM ET AL.: "Improved green fluorescence" cited in the application see the whole document	45
Y	TRENDS IN GENETICS, vol. 11, no. 8, August 1995, ELSEVIER SCIENCE LTD., AMSTERDAM, NL, pages 320-323, XP002029559 D.C. PRASHER: "Using GFP to see the light" see the whole document	45
Y	TIBS TRENDS IN BIOCHEMICAL SCIENCES, vol. 20, November 1995, pages 448-455, XP000606919 CUBITT A B ET AL: "UNDERSTANDING, IMPROVING AND USING GREEN FLUORESCENT PROTEINS" cited in the application see page 451, left-hand column, line 47 - middle column, line 7	45
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## INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 96/19516

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 266, no. 33, 25 November 1991, pages 22075-22078, XP000611463 CHAKRABORTI P K ET AL: "CREATION OF "SUPER" GLUCOCORTICOID RECEPTORS BY POINT MUTATIONS IN THE STEROID BINDING DOMAIN" cited in the application see the whole document ---	49
A	WO 95 07463 A (UNIV COLUMBIA ;WOODS HOLE OCEANOGRAPHIC INST (US); CHALFIE MARTIN) 16 March 1995 see the whole document ---	1-58
A	WO 95 21191 A (WARD WILLIAM ;CHALFIE MARTIN (US)) 10 August 1995 see the whole document ---	1-58
A	NATURE, vol. 369, 2 June 1994, pages 400-403, XP002003600 WANG S ET AL: "IMPLICATIONS FOR BCD mRNA LOCALIZATION FROM SPATIAL DISTRIBUTION OF EXU PROTEIN IN DROSOPHILA OOGENESIS" see the whole document ---	1-58
A	SCIENCE, vol. 263, 11 February 1994, AAAS, WASHINGTON, DC, US, pages 802-805, XP002003599 M. CHALFIE ET AL.: "Green fluorescent protein as a marker for gene expression" see the whole document ---	1-58
P,X	PROC. NATL.ACAD SCI., vol. 93, no. 10, 14 May 1996, NATL. ACAD SCI., WASHINGTON, DC, US; pages 4845-4850, XP002029560 H. HTUN ET AL.: "Visualization of glucocorticoid receptor translocation and intranuclear organization in living cells with a green fluorescent protein chimera" see the whole document ---	1-58
P,X	JOURNAL OF CELL BIOLOGY 133 (5). 1996. 985-996. ISSN: 0021-9525, June 1996, XP000670316 CAREY K L ET AL: "Evidence using a green fluorescent protein- glucocorticoid receptor chimera that the RAN-TC4 GTPase mediates an essential function independent of nuclear protein import." see the whole document -----	29,30, 37,38, 41-46, 48, 51-54,57
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**INTERNATIONAL SEARCH REPORT**

International application No.

PCT/US 96/19516

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: **31-36**  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark:** Although these claims are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/19516

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9507463 A	16-03-95	US 5491084 A AU 7795794 A CA 2169298 A EP 0759170 A	13-02-96 27-03-95 16-03-95 26-02-97
WO 9521191 A	10-08-95	NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)